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# Antibacterial, Anti-virulence factors of *Piper cubeba* extracts on *Escherichia coli* and *staphylococcus saprophyticus* isolated from patient with urinary tract infection

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**Abstract :** Urinary tract infections (UTI ) are between the most common infectious diseases and are among the most common human bacterial infections taking place in both the healthcare and community setting. The present study was carried out to evaluate the antibacterial activities and anti–virulence factors of *Piper cubeba* extract against *Escherichia coli* and Staphylococcus saprophyticus isolated from urine sample of UTI infected patient and identified with the help of colony characterization, gram staining and biochemical testing. In our study, Evaluation the antibacterial activities of three crude extracts was determined by agar well diffusion method. The findings of the present study indicate that the concentration of three extracts exhibit significant differences in their antimicrobial activities against *E. coli* but did not show any antimicrobial activity against *S. saprophyticus* so that excluded from tests of detection effect of extract on virulence factors. MIC determined by using agar well diffusion method, MIC of hot and cold aqueous extract were 25 mg/ml while 50 mg/ml to ethanolic extract. The hot aqueous extract of cubeba at concentration (20%) showed the best action as inhibitor agent against *E.coli*. By detection antivirulence factors of *Piper cubeba* extract against *Escherichia coli* the result showed that *P. cubeba* extract have effect on Serum resistance, Haemagglutination and adhesion Whereas exposure of *E. coli* to cubeba extract did not have any effects on reduced or inhibited the biofilm production. The result of antibiotic susceptibility test showed significant differences at (P between antibiotics that all isolates of *E. coli* were completely resistant (100%) to Ampicillin Whereas (94.2%) to Cefazidime , and ( 82.8%) to Cefotaxime, and (6 5. 7%) to Tetracyclin, and (28.5%) to Imipenem, and (5.7%) of isolates were resistance to Gentamycin and Nitrofuranation While (14.2%) of isolates show intermediate resistance to Amikacin, while effect of *P. cubeba* extract on antibiotic susceptibility show high resistance to all antibiotics used in this study except Gentamicin.
**Key words :** *Piper cubeba* ,virulence factor ,*Escherichia coli*.

## Introduction

Urinary tract infection are one of the most common bacterial infections among the community and hospital acquired infections[1].It affects around 150 million people worldwide each year,

Urinary tract infection is a common contagion among men and women but the incidence is quite high among women due to their physiology[2] Microbial illnesses are depending on the interaction between virulence factors of bacteria and the host and orchestrated by an array of virulence factors that contribute to the pathophysiology and survival of the pathogen in the host continuance of a UTI is influenced by the interaction between the urothelium and the pathogen[3,4], a number of potential virulence factors including adhesins, hemolysin, cell surface hydrophobicity, resistance to phagocytosis, K1 antigen, siderophore,

gelatinase production[5,6,] and motility perhaps responsible for the pathogenicity of uropathogenic *E.coli* ( UPECs).P-fimbriae and curli fibers encourage UPEC adhesion, that is essential in colonization of the host tissues during the first stages of an infection[7]. Although a wide range of pathogenscan cause UTI,*E. coli* are the most pathogens accountable for 80–90 % of community-acquired and 30–50 % of hospital-acquired UTIs[8]Antimicrobial resistance in *E. coli* was reported worldwide and growing rates of resistance with *E. coli* it is a increasing concern in both developed and developing countries[9]. Resistance of bacteria against the traditional antibiotics requests urgent attention to develop a new drug molecules. that well recognized from ancient times that active principles fromplant origin have been used as medicines for diverse diseases and microbial infections[10].Human are using natural products of plants for thousands of years both in the pure forms or crude extracts. 80% of individuals from developed countries used traditional medicine, with plants origin . Recently, a number of studies were conducted in many countries to prove such efficiency. Many plants were used due to their antimicrobial traits, that are mainly synthesized through secondary metabolism of the plant[11]. A broad range of medicinal plants used traditionally were not yet systematically investigated against various microbes[12]. The genus *Piper* of family Piperaceae, with over 1,000 species, is distributed in both hemispheres. A many of searches show that *Piper spp*. was used in traditional medicine since long time for several ailments[13]. *Piper cubeba* is usually known as cubeba , tailed pepper ( due to the stalks attached), kemukus (in Indonesia) and Java pepper (in Java), is a climbing perennial plant[14] *Piper cubeba* was used for a long time as a spice and medicinal plant by traditional healers in diverse places of the world. In Arab traditional medicine, it is used for the treatment of several diseases such as rheumatism, cough and intestinal disorders[15] the fruits of this plant are used as a spice and have medicinal value, often used for the treatment of abdominal pain, diarrhea, chronic bronchitis, asthma, syphilis , gonorrhea, enteritis and dysentery,[16] and it have an inhibitory effect on hepatitis C virus protease[17].

The limited number of publications involving the influence of *P. cubeba* extracts on bacteria and virulence factors encouraged us to carry out research in this area . In the present study, we have evaluated the antibacterial and anti-virulence factors of *P. cubeba* extracts against *Escherichia coli* and *Staphylococcus saprophyticus* that isolated from urinary tract infection patient .

## Material and methods

### Collection and drying of *Piper cubeba:*

*Piper cubeba* were collected from the local market of Babylon province (Iraq) then it was washed thoroughly three times with sterile distilled water. The materials were air dried under hot air oven at 55°C for 3 h and powdered. The powdered samples were hermetically conserved in separate clean container until the time of the extraction.

### Extract preparation

Two different solvent were used in this study ethanol and aqueous(hot and cold water) solvent for prepare three extracts of *P. cubeba* . An amount of 30g. of pulverized fruit was separately soaked in 100 ml of ethanol, and cold sterile distilled water in magnetic stirrer for 24h, while hot aqueous extract prepared by adding 30g of plant material to 100ml of hot water (100 **c**◦) and adjust to magnetic stirrer for 5h . Each preparation was filtered through a sterilized whatman No.1 filter paper[18-20] . Filtered extracts were air dried at 40˚C for 48 h., then stored in labeled sterile bottles in a deep freeze at -18˚C until further use[21].

### Phytochemical analysis of *P .cubeba* :

Three extracts were tested chemically to identify their chemical compounds according to[22]

### Bacterial strain

In this study a total of 150 urine sample were collected from patient suffering from long term urinary tract catheters and urinary tract infection for both genera with age ranging between (11-60) years in Babylon Province, Iraq during a period from September 2015 to February 2016.isolates were identified according to morphology, microscopic examination and biochemical tests. Bacterial cultures were maintained on nutrient broth as a basal medium, supplemented with 15% glycerol. and kept at 4˚C until used[23].

### Antibacterial activity (Agar Well Diffusion Method)

Muller Hinton agar plates were prepared and inoculated with test bacteria by spreading the bacterial inoculums on the surface of the media by the sterile swab. Wells (6 mm in diameter) were punched in the agar by using cork borer. Extracts with different concentrations (2.5% , 5%, 10% , 20% and 40%) were added. The plates were incubated at 37°C for 18 hours. The antibacterial activity was assessed by measuring the diameter of inhibition zone and it recorded in mm[24].

**Determination of minimum inhibitory concentration (MIC**)

MIC defined as the lowest concentration of a extract or drug that completely inhibits the growth of the microorganism in 24 h. The MIC for the hot and cold aqueous and ethanolic extracts against *E. coli*was determined by the modified agar well diffusion method[25]. A twofold serial dilution of each extract was prepared by reconstituting the fruit extract in dilution in sterile distilled water to achieve a decreasing concentration range from 400 mg/mL to 2 5 mg/mL. A 100 µL volume of each dilution was introduced into wells (in triplicate) in the specific media agar plates already seeded with 100 µL of standardized inoculum ( 10cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 C for 24 h and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition , considered as the MIC. [26]

**Antibiotic Susceptibility Test**

Antibiotic susceptibility was determined by the Kirby-Bauer disk diffusion method.Bacterial suspensions were prepared in 1.0 ml of sterile saline solution. 0.5 ml of suspensions was spread on Mueller-Hinton agar plates and then incubated for 24 hours. The inhibition zones were measured in comparison to the Clinical Laboratory Standards institute[27]. The same steps of antibiotics susceptibility test but against bacteria grown with Piper cubeba extract .

**Detection effect of *P. cubeba* extract on bacterial virulence factors**

Virulence factor was detected to *E coli* only because of *Staphylococcus saprophyticus* did not effected by *Pipercubeba* extract, so this study focusing on *E coli* only. and to identify the changes that caused by the extract, *E coli* was grown with MIC concentration of *P . cubeba* at 37 c for 24h in all tests. .

**Serum Resistance:**

This will be studied by using fresh culture of isolates. Overnight cultures *E.coli* grown at $37^0$C on blood agar will be harvested and the cells will be suspended in Hank's balanced salt solution. (HBSS). 0.05 ml of bacterial suspension will be incubated with 0.05 ml of serum at $37^0$ C for 180min. Ten micro litres of samples will be withdrawn and spread on to blood agar plates which will then be incubated at $37^0$C for 18 h and the viable count will be determined. Resistance of bacteria to serum bactericidal activity will be determined by the percentage of bacteria surviving after 180 minutes of incubation with serum in relation to the original count. Bacteria will be termed serum sensitive, if viable count drop to 1% of initial value, and resistant if >90% organisms survive after 180min[28]

**Haemagglutination:**

This will be detected by clumping of erythrocytes by fimbriae of *E.coli* in the presence of D-mannose. The test will be carried out as per the direct bacterial Haemagglutination test-slide method and mannose-sensitive and mannose-resistant haemagglutination tests. The strains of *E.coli* will be inoculated into 1% nutrient broth at $37^0$C for 48 h for full fimbriation. Human blood group 'O' RBCs will be taken and washed thrice in normal saline and made up to 3% suspension in normal saline. They will be used immediately or within a week when stored at 3-$5^0$C. The slide Haemagglutination test will be carried out on a multiple concavity slide. One drop of RBC suspension will be added to a drop of broth culture and the slide will be rocked at room temperature for 5 min. Presence of clumping will be taken as positive for haemagglutination. Mannose-sensitive haemagglutination will be detected by the absence of haemagglutination in a parallel set of test in which a drop of 2% W/V D-mannose will be added to the red cells and a drop of broth culture. MRHA will be detected by the presence of haemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D- mannose [29]

**Detection Biofilm production by Congo Red Agar method:**

A simple qualitative method to detect biofilm production by using Congo Red Agar (CRA) medium. CRA plates were inoculated with test organisms and incubated at 37 for 24 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production. [30]

**Adhesion assay**

**Preparation of the selected isolates of *E .coli* :**

The cells of *E .coli*isolates were centrifugated at 1000 rpm for 20 min, the pellet twice was washed with PBS, and the cells adjusted to contain approximately 1x bacteria/ml by using viable count [31].

**Preparation of epithelial cells:**

Epithelial cells were obtained from the morning urine samples female volunteers (20-30 years) with no history of stone formation or urinary tract infection and had negative urine cultures. The samples were centrifugated at1000 rpm for 5 min. The supernatant was discarded , then the pellet, which has epithelial cells washed with PBS, and centrifugated at 2500 rpm for 10 min Finally the pellet resuspended in PBS to obtain 1x epithelial cell/ ml by using hemocytometer [32].

***In vitro* adhesion test:**

A mixture of bacterial suspension (0.2 ml) and epithelial cells suspension (0.2 ml epithelial cell and 0.1 ml of PBS) were incubated in shaker incubator at 37 degree celcius for one hr. Unattached bacteria were removed by centrifugation three times in PBS at 1000 rpm for 10 min. The final pellet was resuspended in a drop of PBS, then dropped onto a glass slide microscope and air dried. The glass slide was fixed with absolute methanol and covered with Giemsa stain, which used to differentiate epithelial cell and bacterial cell by staining purple and pink, respectively. The number of adherent bacteria on 20 epithelial cells was counted by light microscope [33]. .

## Result and Discussion

In this study a total of 150 urine sample were collected from patient suffering from long term urinary tract catheters and urinary tract infection for both genera with age ranging between11-68 years during a period from September 2015 to February 2016. Identification of pure isolate was done by observing morphological, cultural and biochemical characters according to [34]. The isolates were identified according to [35] and determined it percentage .from 150 urine sample 112 ( 74.6%) specimen give positive growth culture, 104 specimen give pure isolates and 8 specimen was mix ( give 16 isolates ) so the total number of isolates was 120, and number of each bacteria were as follows, *E. coli* was 35( 29.1%), *staphylococcus aureus* 30( 25 .%), *staphylococcus saprophyticus* 8(6.6%), other Gram negative 29(24.1%) and other Gram positive 18(15%) . as show in table (1 )

**Table 1 : percentage of bacteria isolated from urine samples**

| **Bacterial isolates** | **NO of bacteria** | Percentage % |
|---|---|---|
| *Escherichia coli* | 3 5 | 29.1% |
| *Staphylococcus aureus* | 30 | 25% |
| *Staphylococcus Saprophyticus* | 8 | 6.6% |
| *Other Gram negative* | 29 | 24.1% |
| Other Gram positive | 18 | 15% |
| Total isolates | 120 | 100% |
| Total positive growth | 112 | 74.6 |
| No growth | 38 | 25.3% |

The result of this study showed that *E.coli* was the major microorganism among the uropathogens 35(29.1%), and these results were in agreement with the results obtained from other studies conducted

worldwide which approved that *E. coli* is the major pathogen that cause UTIs.[36,37]. Also the results showed a high incidence of UTI in females than males. This difference in occurrence could be due to several clinical factors, including anatomic differences, hormonal effects, and behavioral patterns [38,39]

**Phytochemical screening of *Piper cubeba* :**

After preparation extracts, phytochemical screening of *P. cubeba* done to all extracts .the result of preliminary phytochemical testingshow in table(2)

**Table 2 : phytochemical screening of different*Piper cubeba extracts***

| **Tests** | Aqueous hot | Aqueous cold | Ethanolic |
|---|---|---|---|
| Peptides free amino group test | + | + | – |
| Alkaloid test | – | – | + |
| Flavonoid test | + | + | + |
| Tannins test | ++ | ++ | + |
| Phenols test | ++ | + | + |
| Saponin test | – | – | + |
| Glycoside test | ++ | ++ | + |

(+)positive result ,(++) strong positive result ,.(-) negative result

The aqueous extract contain glycosides, phenols, Flavonoids and tannins only with varying in amount of each compound between the hot and cold extract, whereas the ethanolic, extracts of *P. cubeba* fruits extract contain several chemical compound like glycosides, alkaloids, phenols ,flavonine, tannin and saponine but without free amino group. These results agree with[40], Antibacterial activity of the three crude extracts was determined by agar well diffusion method. The findings of the present study clearly indicate that the concentration of extracts exhibit significant differences at (Pin their antimicrobial activities against *E. coli* but did not show any antimicrobial activity against *S. saprophyticus* so that excluded from the study. The hot aqueous extract of cubeba at concentration ( 20%) showed the best action as inhibitor agent against tested *E. coli* (20mm) (figure1), followed by the cold aqueous (15mm) and ethanolic(12mm), antibacterial activity of *P. cubeba* extracts was show in table (3).

**Table 3: Mean antibacterial activity of *P. cubeba* fruit extracts against bacterial pathogens determined by diameter of inhibition zone in (mm.)**

| Concentration of extracts | Type of extracts | Inhibition zone in (mm) Mean± SD | |
|---|---|---|---|
| | | ***E. coli*** | ***S. saprophyticus*** |
| **10%** | Hot aqueous extract | 17±1 | - |
| | Cold aqueous extract | 13.33± 0.57 | - |
| | Ethanolic extract | 8.33±0.57 | - |
| **20%** | Hot aqueous extract | 20.66 ± 2.08 | - |
| | Cold aqueous extract | 15.33 ± 1.57 | - |
| | Ethanolic extract | 13 ± 1 | - |
| **40%** | Hot aqueous extract | 20 ± 3 | - |
| | Cold aqueous extract | 15 ± 2 | - |
| | Ethanolic extract | 13 ± 2 | - |

(-) NO inhibition, SD=Stander division, Influence of concentration LSD(0.05)=0.507,Influnce of type of extract =NO significant

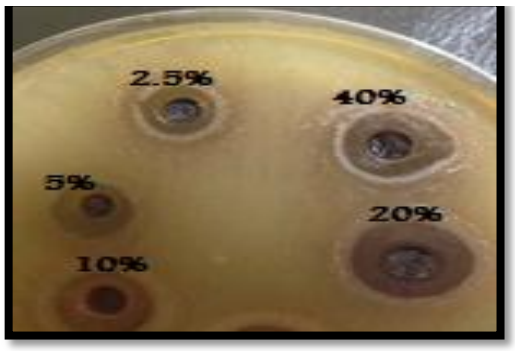



**Figure 1 :Antimicrobial activity of hot aqueous extract of P.cubeba on E coli**

This result were in agreement with those reported by [41] it was found that water extract showed inhibition activity against *E.coli* (15mm)and *P. aeruginosae* followed by ethanol (10mm), acetone and chloroform extract , All the oil present in Piper are mostly phenolics and are antibacterial because they interfere with selective permeability of cell membrane but these terpens cannot discriminate between human cell membrane and microbial cell membrane, so cannot be used against systemic infection ,therefore it was though worthwhile to see the in vitro effect of extract of piper species[42]phenol compound has precipitate activity on microbial enzyme and leading to inhibit and lost their function[43] while hydroxyl group in flavonoids have ability to composite with cell wall proteins and break down the cell membrane of bacteria.[44].MIC of *P. cubeba* fruit extracts against *E. coli* determined by (mg/ml) the MIC to hot and cold aqueous extract were 25 mg/ml while 50 mg/ml to ethanolic extract as shown in Table ( 4).

**Table 4 : Minimum inhibition concentration of *P . cubeba* extracts on *E.coli***

| Minimum inhibition concentration | | |
|---|---|---|
| **Hot aqueous extract** | **Cold aqueous extract** | **Ethanolic extract** |
| 25mg/ml | 25mg/ml | 50mg/ml |

**Antibiotic susceptibility :**

The effects of different antibiotics on bacterial isolates were investigated and the antibiotic sensitivity was measured depending on a diameter of inhibition zone (mm) according to [27] the antibiotics susceptibility patterns of *E coli* isolates were varied as shown in table ( 5)

**Table 5 : Antibiotic susceptibility tests of *E .coli* isolates**

| No | Antibiotic | Sample | Inhibition zone mean ± SD | Sensitive No.(%) | Intermediate No(%) | Resistance No.(%) |
|---|---|---|---|---|---|---|
| **1** | Amikacin | Ak (30µg) | 18.9714 ±2.55500 | 30(8 5.7%) | 5(14.2%) | _ |
| **2** | Ampicillin | AM (10µg) | 0.0 ± 0.0 | _ | _ | 35(100%) |
| **3** | Ceftazidime | CAZ ( 30µg) | 8.6857 ± 2.47026 | _ | 2( 5.7%) | 33(9 4.2 %) |
| **4** | Gentamicin | CN(10 µg) | 15.5905 ±2.54458 | 29(8 2. 8%) | 4 (1 1.4%) | 2(5.7%) |
| **5** | Imipenem | IP(10 µg) | 19.3905 ±2.66565 | _ | 25 (71.4%) | 10(28.5%) |
| **6** | Nitrofloranation | F(300 µg) | 17.1143 ±2.78497 | 18(5 1.4%) | 15(4 2.8%) | 2(5.7%) |
| **7** | Tetracyclin | TE(30 µg) | 11.8286 ±3.77353 | 12(34.2%) | _ | 23(65.7%) |
| **8** | Cefotaxime | FOX(30µg) | 10.8476 ±2.46819 | _ | 6(17.1%) | 29( 82.8%) |

(-) Negative result,= LSD(0.05)=0.602

In present study statistical analysis to antibiotics susceptibility test showed significant differences at (p between antibiotics, These results were agreed with locally study done by[45]. The resistance Time of Ampicillin Cefotaxime and Ceftazidime may be due to increased consumption of these antibiotics, self-medication, and transfer of resistant[46].in present study isolates were showed the highest sensitivity to antibiotics such as Amikacin ( 85. 7%), Gentamicin (8 2. 8%), these result of current study were in similar with those done by [47]. Our results revealed that Amikacin was the most prescribed empirical antibiotic followed by Gentamicin. The results of this study also support the recommendation of the aminoglycoside, amikacin as suitable antibiotic for treating uropathogenic *E. coli* infections.(figure2)

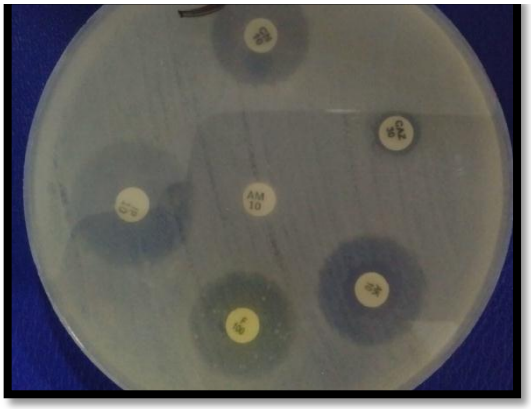


**Figure 2 : Antibiotic susceptibility of *E coli***

**Effect of MIC concentration of *Piper cubeba* extract on antibiotics susceptibility of *E.coli***

Antibiotic susceptibility test was studied for *E.coli* after grown with MIC concentration of hot aqueous extract (25gm/ml) of *P. cubeba* to determined influence and interaction between them. Firstly bacteria was grown with the extract at 37c for 24h, then same steps of antibiotic susceptibility test was done. Obtained result showed resistance of bacteria against Ceftazidime Nitrofuranation, Tertracyclin, Amikacin and Cefotaxim was increased as show in table ( 6)

**Table 6: Effect of *P. cubeba* extracton Antibiotic susceptibility**

| No | Antibiotic | Sample | Sensitive No.(%) | Intermediate No(%) | Resistance No.(%) |
|---|---|---|---|---|---|
| **1** | Amikacin | Ak (30µg) | 18(51.4) | 17(485) | - |
| **2** | Ampicillin | AM (10µg) | - | - | 35(100%) |
| **3** | Ceftazidime | CAZ( 30µg) | - | - | 35(100%) |
| **4** | Gentamicin | CN (10 µg) | 29(8 2. 8%) | 5 (14.2 %) | 1(2.8%) |
| **5** | Imipenem | IP(10 µg) | - | 11 ( 31.4%) | 24(68.5%) |
| **6** | Nitrofloranation | F(300 µg) | 5 (14.2%) | 9( 25.7 %) | 21( 60%) |
| **7** | Tetracyclin | TE(30 µg) | 3( 8.5%) | 7(20%) | 2 5(71. 4%) |
| **8** | Cefotaxime | FOX(30µg) | - | - | 35( 100%) |

The result showed that the sensitivity of all antibiotics have been decreased except Gentamicin it remained without changes .that may be caused by interference and antagonism between the extract ( due to compounds of *Pipercubeba*) and antibiotics**.** By using *vitek* systems 2 of antibiotic susceptibility it was found *E. coli* (without extract )resist to Ampicillin ,.Gentamicin and Ceftazidime and sensitive to Imipenim , whereas bacteria grown with the extract showed resistance to Ampicillin and Ceftazidime and were sensitive to Imipenim and Gentamicin. These result reveal differences in Antibiotics susceptibility between disc diffusion method and Antibiotics susceptibility by using *vatic* system. (figure3)

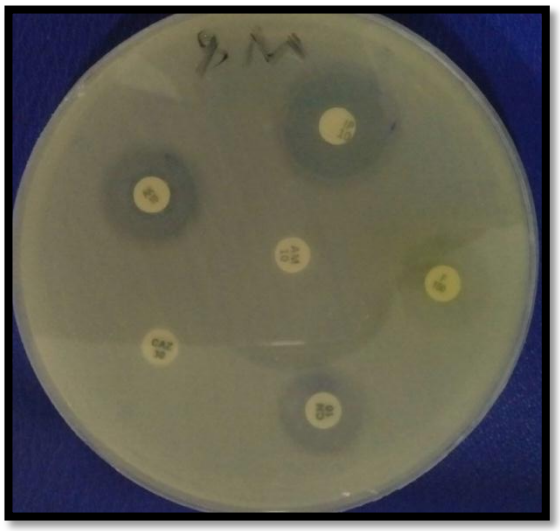


**Figure 3 :Effect of MIC hot *P. cubeba* extract on antibiotics susceptibility of *E. coli***

**Detection effect of *Piper cubeba* extract on virulence factors of *E. coli***

**Serum resistance:**

is the property by which the bacteria resist killing by normal human serum due to the lytic action of the alternative pathway of complement system [48] In the present study, 33 (94.2)% of the isolates were resist to serum bactericidal activity. Our study results were comparable with study done by [49] where they found that 93% of the isolates were resistant to serum bactericidal activity, whereas only four percent were serum resistant among control strains in compared, while the result of serum resistance after treated with cubeba extract showed 19(57.5%) from these 33 isolates decreased in viable bacterial number and conceder intermediate in their resistance According to the method of[50] these effect may be accrue due to changing in the number or structure of enveloped compounds that protect bacteria from lysis or killing by normal human serum

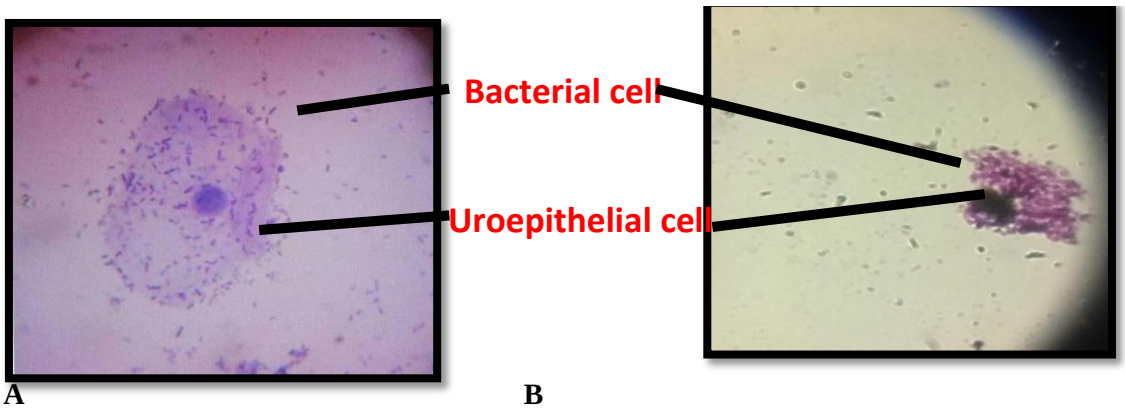
**Haemagglutination:** is mediated by fimbriae[51] Virulence determinants such as P fimbriae and type 1 fimbriae are more frequent in UPEC The host cell receptors for P fimbriae are globoseries of glycosphingolipids (GSLs), which are expressed on uroepithelial cells. These receptors are abundant on the uroepithelial[52] in the present study10 ( 28.5 % )of *E coli* isolates was MRHA ,and these result agree with the studies of[46,50] . present study showed that plant extract inhibited erythrocyte haemagglutination by uropathogenic *E. coli* . which indicates the dysfunction of P fimbriae, as It is known that *P. cubeba* are rich in tannins compounds with the structure very similar to receptors found on bladder and kidney cells[53] Therefore, these compounds act by binding to fimbriae and thereby preventing their attachment to the host tissue. According to the results obtained in our study (4) *E coli* isolates from (1o ) MRHA lost their ability of haemagglutination when incubated with *P. cubeba* extract.[54] showed that some of medical plants used in their study inhibited erythrocyte haemagglutination by uropathogenic *E. coli* strain and that depended on the type and concentration of the plant extracts used .

**Biofilm production**: the result were 35(100%)positive for Biofilm. *E coli* that treated with cubeba extract had been cultured on Congo red agar plates. The current study indicate that the exposure of *E. coli* to plant extract haven't any effects on reduction or inhibition the biofilm. in other study done by[55]on the effect of medical plant extract it was shown that medical plant reducing biofilm formation after 1-10 days of incubation and that depended on plant species and concentration of extracts (figure4) .

**(A)** **(B)**

**Figure 4 : biofilm production by Congo red plate method.(A)*E.coli* without extract,(B)with hot P.cubeba extract**

**Bacterial adherence** not only contributes to colonization but also to invasion, biofilm formation, and host cell damage.[56] Adhesion of *E coli* isolates to uroepithelial cell have been studied ,and in presence and absent of *P. cubeba* extract the result show decreasing number of bacteria that adhere to uroepithelial cell, number of bacteria became between (10- 15 bacteria/ cell) while bacteria without extract show a high adhesion ability (20- 30 ).[57] who reported the adhesion of the Chinese herb- susceptible *E.coli* to human uroepithelial cells was significantly lower compared with that of the resistant E. coli . the increase in adhesion of resistant *E. coli* culturing in herbal solution can theoretically be caused by several mechanisms, such as the maintenance of fimbriae, which need further investigation[58].(figure5)




**A** **B**

**Figure 5 : Adhesion of *E.coli* to uroepithelial cell of human(A) Before treated with the extract ,(B) after treated with the hot P.cubeba extract**

## Conclusion

All plant extracts used in our study showed antibacterial activity but hot Aqueous extract was the best *P. cubeba* extract altered virulence factors (Hemolysin production, serum resistance, haemagglutination and adhesion to uroepithelial cell of human) in examined bacteria. Detection of effect *of P. cubeba* extract at MIC concentration on antibiotics concentration showed increased resistance of most of antibiotics used in this study . That may be due to interference and antagonism (due to compounds of *Pipercubeba*) with antibiotics**.** Therefore, our results should be confirmed in clinical trials to be able to recommend tested plant extracts in prevention of and treatment of UTIs.

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